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THE EFFECT OF VITAMIN A AND HYDROCORTISONE ON THE NORMAL ALKALINE PHOSPHATASE RESPONSE TO SKIN WOUNDING IN RATS*

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INTRODUCTION

The histochemical distribution of alkaline phosphatase activity has been studied in normal and wounded skins of rats (1–4), guinea pigs (2, 5, 6) and man (7–9) and the activity of this enzyme has been associated with fibrous protein synthesis (1, 5, 10).

Normally, mammalian skin contains only trace amounts of alkaline phosphatase located in the stratum granulosum, hair follicles, sebaceous glands and some connective tissue cells (4, 7, 9). During wound healing, however, an increase in the phosphatase reaction is noted in the keratohyalin granules of the stratum granulosum associated with keratinization and in connective tissue at the time of maximum collagen formation. The presence of the enzyme at these sites suggested a relationship between the keratin-collagen synthesis and alkaline phosphatase activity (1, 5, 10). In addition, Danielli, Fell and Kodicek (5) demonstrated in scorbutic guinea pigs, that both wound healing and the phosphatase reaction were severely retarded and these phenomena paralleled the degree of vitamin C deficiency.

The purpose of the present study was to evaluate the tensile strength and alkaline phosphatase activity during the healing process in control wounded rats and animals treated with vitamin A and hydrocortisone. Large doses of vitamin A in animals has been reported to stimulate bone growth while a deficiency retards osteogenesis and causes epithelial metaplasia (11). On the other hand, the local or systemic administration of cortisone or hydrocortisone acetate significantly depresses scar tissue formation and wound tensile strength (12–14).

MATERIALS AND METHODS

Albino rats (Carworth Farms), weighing an average of 200 gm were divided into 4 treatment groups as follows: 1) control, 2) vitamin A, 3) hydrocortisone and 4) vitamin A + hydrocortisone. In addition, fifteen, normal, intact rats were included for evaluation of basal enzyme levels.

The hair was removed from the dorsal surface with an electric clippers and the skin wounds were incised under light ether anesthesia. The midline incision was approximately 1½" long and located 2" below the interscapular region. The 50 mg vitamin A acetate and/or 5 mg hydrocortisone acetate (Merck, Sharp and Dome) were locally applied along the full extent of the incision and the wounds were closed with stainless steel clips.

The tensile strength was determined on ten animals from wounded treatment groups on 4, 7, 10 and 14 days according to a previously published procedure (12). Five other rats were killed by decapitation at 0.25, 0.50, 0.75, 1, 2, 4, 7, 10, 14, 17, 20 and 26 days. The wounds were rapidly removed by cutting 3.5 mm on either side of the incision (total width 7 mm). A section of skin from the same dorsal area was taken from the normal, intact, untreated animals. After weighing on an analytical balance a piece of the excised tissue (excluding the areas of the stainless steel wound clips) was quenched between two blocks of dry ice for 1 minute. The frozen skin was placed in a rubber finger cot and shattered by hitting it several times with a hammer. The macerated tissue was transferred to a flask containing 10 ml of cold distilled water and homogenized with a Vir Tis No. 45 homogenizer until a uniform suspension was obtained (approximately 5 minutes). The homogenate was then diluted to a specific concentration (1%) and aliquots used in the alkaline phosphatase procedure according to Manning, Steinetz, Babson and Butler (15). Ten units of alkaline phosphatase activity liberates 1 mg of phenolphthalein from phenolphthalein diphosphate in 1 hour at pH 9.6 and 37° C under standard conditions.

Dry weights were obtained by drying a piece of the wounded skin in a vacuum oven at 60° C for 24 hours.

The biochemical data was evaluated statistically by analysis of variance and the "F test" for significance (16). The mean and standard errors were calculated for the tensile strength results and submitted to the "t tests" of Student.

RESULTS AND DISCUSSION

The data in Table 1 indicate that wounding, per se, resulted in enhanced phosphatase

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levels at all time periods tested, relative to the normal intact skins (11.26 units ± 1.04).

During the normal healing process, biochemically determined alkaline phosphatase demonstrated two peaks; at 12 hours during eschar formation and around day 10 corresponding with collagen synthesis (Fig. 1). The activity at day 26 was similar to the intact control and these results are comparable to earlier histochemical reports (1, 4). Relative to the wounded controls, vitamin A and the combined treatment increased whereas hydrocortisone decreased significantly the first peak in the alkaline phosphatase reaction (Fig. 1 and Table 1). In animals receiving vitamin A + hydrocortisone the second phase was 2.5x greater while animals treated with these compounds individually, demonstrated an alkaline phosphatase response similar to the controls (Table 1). With hydrocortisone, however, the

TABLE 1

The average (±S.E.) alkaline phosphatase levels of control and treated, wounded rats over the 26 day period*

Treatment	Time (days)											
	0.25	0.50	0.75	1	2	4	7	10	14	17	20	26
Control	23.0 ± 1.3	73.2 ± 19.2	33.0 ± 2.7	26.1 ± 3.5	22.6 ± 1.2	30.6 ± 6.9	30.0 ± 2.6	41.4 ± 3.1	29.2 ± 3.1	30.0 ± 2.6	24.2 ± 1.1	12.0 ± 2.3
Vitamin A	41.8 ± 6.3	121.8* ± 20.3	93.6† ± 13.6	98.6† ± 13.7	86.4† ± 12.1	49.4 ± 3.4	50.4 ± 9.5	45.8 ± 4.1	35.4 ± 2.3	35.2 ± 6.7	25.4 ± 1.8	11.8 ± 2.0
Hydrocortisone	23.6 ± 2.0	43.8‡ ± 5.9	44.2 ± 7.0	34.2 ± 7.1	25.8 ± 3.4	26.6 ± 3.0	30.2 ± 1.2	40.4 ± 0.7	46.2 ± 1.7	49.4 ± 6.1	52.6† ± 6.8	26.2 ± 3.1
Vitamin A + Hydrocortisone	21.4 ± 4.2	47.8‡ ± 4.4	82.6† ± 20.1	54.2† ± 9.5	50.6† ± 11.5	41.8 ± 2.3	46.6 ± 5.0	103.2† ± 18.0	44.6 ± 0.5	42.6 ± 2.2	39.0 ± 6.9	24.4 ± 2.2

* Expressed in units/gm dry tissue. Ten units of alkaline phosphatase activity liberates 1 mg phenolphthalein from phenolphthalein diphosphate in 1 hr at pH 9.6 and 37°C under standard conditions.

† Significantly greater than control $p < 0.05$.

‡ Significantly less than control $p < 0.05$.

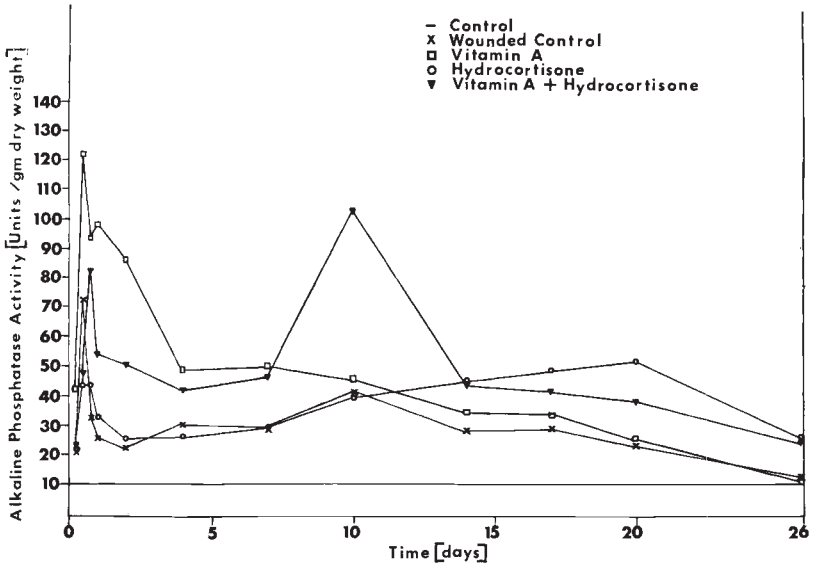

FIG. 1. The alkaline phosphatase activity during the healing process in wounded control rats and animals treated with vitamin A and hydrocortisone.

second peak of phosphatase activity was delayed to day 20 (Fig. 1).

The role of alkaline phosphatase in wound healing is obscure, although its presence at high levels coincident with other biochemical events, certainly implicates this enzyme in the epidermal and dermal regeneration processes. The first peak in the phosphatase reaction (12 hours) which is associated with the invading leucocytes of the eschar, may be involved in catabolic mechanisms for the removal of dead cells and other tissue debris. If one considers the hydrolytic activity of alkaline phosphatase on carbohydrate phosphates, anabolic processes are indicated as well, since increased deposition of glycogen and production of nucleic acids and mucopolysaccharides is noted at this time (10, 17–22). It is of interest that the "productive" and "collagen phases" of wound healing as suggested by Dunphy and Udupa (18) corresponds to our peaks in alkaline phosphatase activity.

As demonstrated by Williamson and Guschlbauer (21, 22) and Guschlbauer and Williamson (19), the synthesis of RNA and DNA is maximal 8 days after wound initiation. These same investigators reported that the phosphate pool was essentially unchanged although the turnover rate was significantly increased; thus, the second phase in the alkaline phosphatase response to wound healing may be necessary to maintain the level of the phosphate pool. With enhanced formation of glycogen (energy source), mucopolysaccharide (homogeneous matrix necessary for collagen deposition) and RNA (protein synthesis), the conditions necessary for fibrous protein production (keratinization and collagen formation) are maximal around the eighth day of wound healing.

If the enzymatic results are compared with tensile strengths (Fig. 2 and Table 2), one is immediately aware that no direct correlation is possible since the wound strengths were similar in vitamin A and control animals and lowered in rats receiving hydrocortisone and the combined treatment. Apparently, the events for the production of tensile strength are so complex and interrelated, that the true correlation of it with alkaline phosphatase is completely obscured. For example, Perumal, Samy, Lakshuanan, Jungalwala and Rao (23), Wolbach (24), Wolf and Varandani (25) and Wolf (26) demonstrated the necessity of vitamin A for the synthesis of mucopolysaccharides. If the hypothesis presented in this communication is reasonable, one might expect enhanced mucopolysaccharide formation, alkaline phosphatase activity and tensile strength in vitamin A-treated wounded animals. On the other hand, hydrocortisone administration, which decreases scar tissue formation and tensile strength should also depress the phosphatase reaction during the collagen phase. In contrast, however, neither the tensile strength (except at day 4) was increased in the former case nor the alkaline phosphatase activity depressed in the latter one.

A reasonable explanation for these phenomena is that the sequence of events is so critically controlled in relation to quantities and types of substrates present, time, etc. that an increase in any one component does not necessarily result in enhanced would healing. The reverse is not true, however, since a deficiency in a critical element will most probably lead to an abnormal healing process.

SUMMARY

Alkaline phosphatase activity and tensile strength was evaluated duing the healing process in wounded control rats and animals treated with vitamin A, hydrocortisone and vitamin A + hydrocortisone. Peaks in the phosphatase reaction were noted at 12 hours and ten days after wound initiation in control animals. Vitamin A alone or in combination with the glucocorticoid increased, while hydrocortisone decreased, significantly the first phase of the enzyme activity. The second peak was enhanced 2.5 x by vitamin A + hydrocortisone, while rats receiving these compounds individually, demonstrated an alkaline phosphatase level similar to the controls. With hydrocortisone alone the second phase of phosphatase activity was delayed to day 20.

Wound tensile strength, assessed at days 4, 7, 10 and 14 was similar in control and vitamin A animals. In contrast, the glucocorticoid significantly lowered the tensile strength and this effect was only partially reversed by vitamin A. No direct correlation could be made between the alkaline phosphatase activity and wound tensile strength.

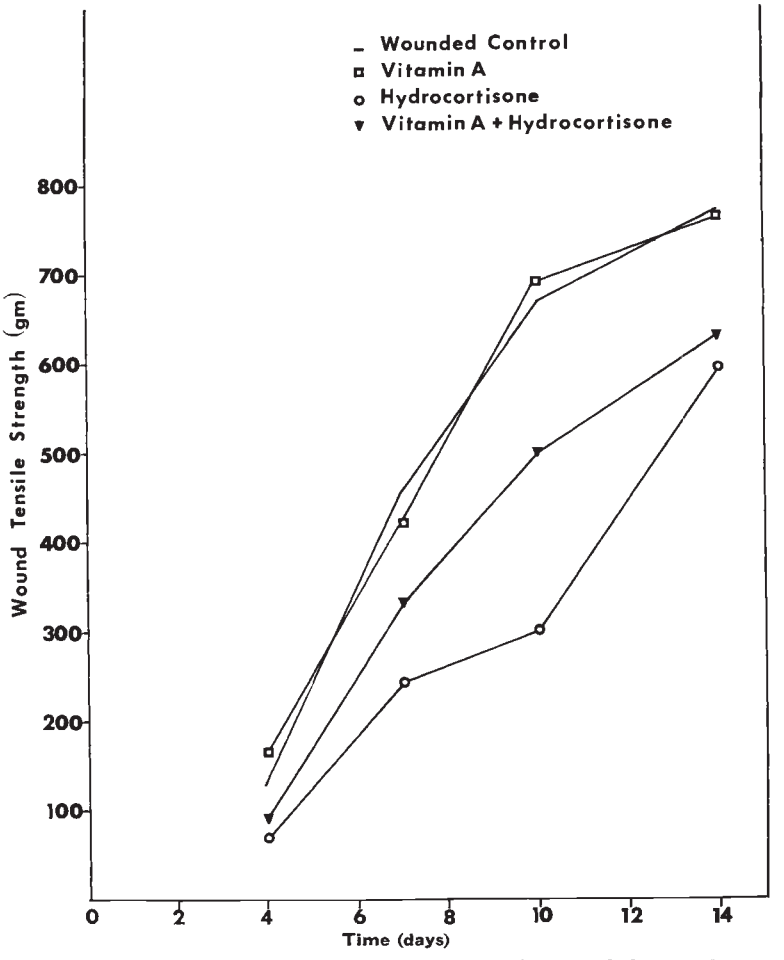

FIG. 2. The tensile strength during the healing process in wounded control rats and animals treated with vitamin A and hydrocortisone.

TABLE 2

The average (±S.E.) tensile strength of control and treated, wounded rat skins*

Treatment	Time (days)			
	4	7	10	14
Control	129 ± 9.5	453 ± 58.5	668 ± 16.7	772 ± 56
Vitamin A	165 ± 6.1†	419 ± 20.8	690 ± 30.8	763 ± 30
Hydrocortisone	68 ± 4.3‡	246 ± 30.0‡	300 ± 26.9‡	594 ± 31‡
Vitamin A + Hydrocortisone	91 ± 3.0‡	330 ± 23.0‡	497 ± 29.2‡	630 ± 42‡

* gm.

† Significantly greater than control $p < 0.01$.

‡ Significantly less than control $p < 0.01$.

The alkaline phosphatase results are discussed in relation to the formation of fibrous proteins (collagen and keratin) during the healing process.

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